Sanger Sequencing - Sample Submission Guidelines

Plasmid Samples

1. About 200 ng plasmid DNA is used in one Sanger sequencing reaction, the maximal volume of plasmid DNA used is 5 ul. We prefer plasmid with minimum concentration of 30 ng/ul.
2. If the plasmid size is more than 20 kb, please double the amount of DNA.

PCR Samples, Purified or Unpurified

1. We accept both purified and unpurified PCR samples. If samples are unpurified PCR, we will perform PCR purification before sequencing.
2. For PCR product, we use about 10 ng DNA per kb, so please provide PCR size information in your order.
3. Note: Prior to submission, it is recommended to confirm by agarose gel electrophoresis that specific PCR products are present without undesired bands.

E. coli Colonies

1. We accept agar plates, colony suspensions, and overnight cultures.
2. One of three methods are used to amplify DNA before sequencing. PCR is the most popular option, since this allows next day data delivery; RCA and plasmid miniprep require an extra day.
   - ✓ PCR (please provide amplicon size, forward and reverse primer)
   - ✓ Rolling Circle Amplification (RCA)
   - ✓ Plasmid miniprep (please provide antibiotics information)
3. For bacterial suspension, place all samples in either tightly sealed 8-strip tubes or strip capped 96-well plate.

Genomic DNA

1. We perform PCR amplification prior to sequencing, so please provide PCR primers and expected amplicon size.
2. Please send at least 10 ul of DNA sample, with concentration ≥ 30 ng/ul.

Sequencing Primer

We use 1 ul of 3 uM primer in 1 sequencing reaction and accept primer at 3 - 100 uM (please provide primer concentration with your sample submission)

Free Primers

Available Common Primers
Complete List in Excel || Search Online

Cost per sanger sequencing reaction is $5 if templates are plasmid DNA or purfied PCR. Extra cost is applied for other samples types (genomic DNA, colony, RCA, and unpurified PCR). Volume based discount is available, please email support@poochonscientific.com for discount or quotation.com for discount or quotation.

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Premixed, DNA and Primer in Same Tubes

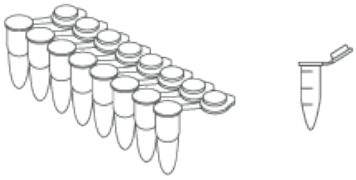
Premixed for success!

Advantages of premixed samples:

- ✓ Track samples easily
- ✓ Get results faster
- ✓ Enjoy low cost per reaction
- ✓ Improve success rate significantly

Premix	10 µl Template		2 µl Primer
Sample	Type & Size	Concentration	Concentration
Purified Plasmid	ds plasmid DNA ≤ **10 kb**	80 ng/µl	**5 pmol/µl (5 µM)**
OD260/280 ≥ 1.80 and ≤ 1.95	ds plasmid DNA ≥ **10 kb**	100 ng/µl	
	ss plasmid DNA ≤ **10 kb**	50 ng/µl	
Purified PCR	≤ 1 kb	5 ng/µl	**5 pmol/µl (5 µM)**
	1 kb - 2 kb	10 ng/µl	
	1 kb - 2 kb	15 ng/µl	
	1 kb - 2 kb	20 ng/µl	
	> 4 kb	50 ng/µl	

Unpremixed, DNA and Primer in Separate Tubes



Tube 1: Template

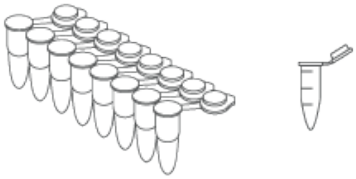
Tube 2: Primer (5 pmol/µl or 5 µM), 10 µl. Or, use our free primer library.

Primer For Sequencing:
Preferred Tm: 50-60°C, GC content: 40-60%

10 µl Template		
	Type & Size	Concentration
Plasmid	ds plasmid DNA (≤ 10 kb)	80 ng/µl
	ds plasmid DNA (> 10 kb)	100 ng/µl
	ss plasmid DNA (< 10 kb)	50 ng/µl
PCR product (purified or unpurified)	≤ 1 kb	5 ng/µl
	1 kb ~ 2 kb	10 ng/µl
	2 kb ~ 3 kb	15 ng/µl
	3 kb ~ 4 kb	20 ng/µl
	> 4 kb	50 ng/µl

E.coli Colonies

Pick a colony and resuspend in diH2O or Tris Buffer

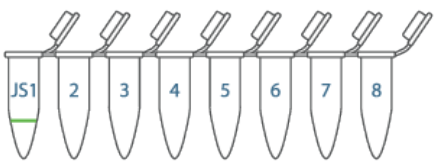


Tube 1: Colony Suspension **Tube 2:** Primer

1. Colonies need to grow for at least 16 hrs at 37°C to reach good visible size
2. Pick a single colony with sterile tip and re-suspend in 30 ul sterile diH2O or Tris buffer (10mM, ph8.0), 15 ul will be submitted to us, and the rest 15 ul will be kept by clients for future uses
3. Prepare 2 separate tubes: 1 contains 15ul colony suspension, 1 contains 5 ul primer at 5pmol/ul

How to Label Sample Tubes

Label the samples with your initials followed by numbers - Example: Samples from John Smith



8-strip PCR tube for samples containing template

Label on the cap as well as on the side of the tube